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Povidone-Iodine (PVI) has a profound effect on in vitro osteoblast proliferation and metabolic function and inhibits their ability to mineralise and form bone

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The device(s)/drug(s) that is/are the subject of this manuscript is/are not FDA-approved for this indication and is/are not commercially available in the United States.

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Structured Abstract

Study Design. A study examining the clinical protocol of scoliosis wound irrigation, demonstrating Povidone-Iodine's (PVI) effect on human osteoblast cells. Primary and immortal cell line osteoblasts were treated with 0.35% PVI for 3 minutes, and analyzed for proliferation rate, oxidative capacity and mineralisation.

Objective. To model spinal wound irrigation with dilute PVI *in vitro*, in order to investigate the effect of PVI on osteoblast proliferation, metabolism and bone mineralisation.

Summary of Background Data. Previously PVI irrigation has been proposed as a safe and effective practice to avoid bacterial growth following spinal surgery. However, recent evidence in multiple cell types suggests that PVI has a deleterious effect on cellular viability and cellular function.

Methods. Primary and immortal human osteoblast cells were exposed to either PBS control or with 0.35% PVI for 3 min. Cellular proliferation was measured over the duration of 7 days by MTS assay. Oxygen consumption rate (OCR), extracellular acidification rate (ECAR) and proton production rate (PPR) were analysed using a Seahorse XFe24 Bioanalyzer. Protein expression of the electron transport chain subunits CII-SDHB, CIII-UQR2 and CV-ATP5A were measured via Western blotting. Mineralised bone nodules were stained with alizarin red.

Results. Expressed as a percentage of normal osteoblast proliferation, osteoblasts exposed to 0.35% PVI exhibited a significant 24% decrease in proliferation after 24 h. This was a sustained response, resulting in a 72% decline in cellular proliferation at 1 week. There was a significant reduction in OCR, ECAR, and PPR ($p < 0.05$), in osteoblasts that had been exposed to 0.35% PVI for 3 min, coupled with a marked reduction in the protein expression of CII-SDHB. Osteoblasts exposed to 0.35% PVI exhibited reduced bone nodule mineralisation compared to control PBS exposed osteoblasts ($p < 0.01$).

Conclusion. PVI has a rapid and detrimental effect on human osteoblast cellular proliferation, metabolic function, and bone nodule mineralisation.

Key Words: Povidone-Iodine, PVI, Osteoblasts, wound irrigation, Spinal Surgery, Bone Mineralization, Cellular Metabolism, Cell Viability, Cell Proliferation

Level of Evidence: N/A

Key Points

- Acute exposure to dilute PVI markedly reduces human osteoblast proliferation.
- There is a significant reduction in human osteoblast metabolic capacity (OCR, ECAR, and PPR) after exposure to 0.35% PVI for 3 min.
- Human osteoblasts exposed to 0.35% PVI for 3 min exhibited a marked reduction in succinate dehydrogenase B (CII-SDHB) protein expression.
- Osteoblasts exposed to 0.35% PVI exhibited reduced bone nodule mineralisation.

Mini Abstract

This study modelled the effect of using PVI wound irrigation during spinal surgery on human osteoblast cells. Osteoblasts treated with 0.35% PVI for 3 minutes exhibited a decrease in proliferation rate, a significant reduction in metabolic capacity, and reduced bone nodule mineralisation.

1 Introduction

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2 Surgical wound infection is a serious complication following spinal surgery. Infection rates in spinal surgery vary hugely and are dependent on a plethora of factors including patient co-morbidities and type of surgery being performed. In particular deformity correction procedures, with long wounds, long operating times and medical co-morbidities; infection rates can be as high as 19%. Surgical site infections can progress to deep infection, incur multiple operations with increased healthcare costs and impaired patient outcomes (1-6).

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To reduce the risk of wound infections, many spinal surgeons are using intra-operative antimicrobial wound irrigates (7). One such irrigate which has been advocated and utilised is dilute Povidone-Iodine (PVI) (8). However, there is a paucity of evidence supporting this off-label practice. Two clinical papers have previously described this as a safe and effective practice (8, 9) but there is evidence that PVI has a deleterious effect on cellular viability and cellular function. Studies across multiple cells types and tissues, including fibroblasts, keratinocytes, chondrocyte, immune cells and synovial tissue have now demonstrated that PVI has cytotoxic effects (10-13).

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The PVI wound irrigation technique, as described by Chang et al. (9), involves complete immersion of the wound for 3 minutes with a preparation of 0.35% of PVI (diluted from commercially available 10% PVI "Videne"), followed by copious irrigation with normal saline, prior to decortication and wound closure.

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25 Two laboratory studies (one human, one animal) describe the effects of PVI on
26 osteoblasts. Whilst results were heterogeneous, all described a toxic effect of PVI on
27 the osteoblast (14, 15).

28
29 From a spinal surgery perspective, there are no studies which have examined
30 whether PVI at clinically advocated concentrations has deleterious effects on human
31 osteoblast viability and function. Addressing this is imperative since osteoblast
32 proliferative and metabolic activity, together with their ability to form bone and
33 mineralise, is essential for the establishment of solid fusion post-surgery.

34
35 The aim of this study was to model the clinical protocol *in vitro*, in order to investigate
36 the effect of dilute PVI washout on osteoblast proliferation, metabolic function and
37 osteoblast bone nodule formation and mineralisation.

38 **METHODS**

39 **3.1 Cell culture**

40 Primary human osteoblast cells were cultured in differentiation media (10% fetal calf
41 serum (FCS), penicillin (100 units/ml), streptomycin (100µg/ml), L-glutamine (2mM),
42 non-essential amino acids (1%, Invitrogen) and Amphotericin B (2.5 µg/ml), β-
43 glycerophosphate (2mM), Ascorbic acid (50µg/ml) and Dexamethasone (10nM))
44 entirely. The human osteoblast cell line hFOB 1.19 (ATCC® CRL-11372™) (ATCC,
45 England, UK) was cultured in growth media (1:1 mixture of Ham's F12 Medium
46 Dulbecco's Modified Eagle's Medium, with 2.5 mM L-glutamine (without phenol red),
47 10% fetal bovine serum (FBS), and 0.3mg/ml G418 prior to differentiation media.

49 **3.2 Preparation of primary human osteoblasts**

50 Ethical approval was granted by the United Kingdom (UK) National Research Ethics
51 Service (National Health Authority, reference NRES 14-ES-1044), and institutionally
52 approved and sponsored by [REDACTED] as required under the UK
53 Research Governance Framework. Study participants were provided in advance with
54 a participant information sheet, and a participant consent form. Following patient's
55 written consent, the femoral head was collected from a female patient (aged 62
56 years) undergoing total joint replacement surgery for hip osteoarthritis. The articular
57 cartilage was removed from the femoral head and the subchondral bone cut into
58 small chips. The bone chips were then washed thoroughly in serum-free primary
59 osteoblast media to remove any excess blood, connective or adipose tissue and
60 then incubated in differentiation media in a culture flask at 37°C (5% CO₂).
61 Differentiation media was replaced with fresh media 2x per week, and the bone chips
62 removed upon the appearance of osteoblast cells.

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3.3 Osteoblast proliferation assay

Primary human osteoblasts and the human osteoblast cell line (hFOB 1.19) were plated at 6×10^3 cells per well in a 96 well plate. At confluency, the cells were incubated with either PBS control or with 0.35% PVI (diluted in PBS) for 3 minutes at room temperature. The wells were then aspirated, washed 5x with PBS and then placed back into osteoblast growth media. After 24, 48, 120, 144 and 168 h at 33°C, an MTS (Cell Titer Aqueous One Solution Cell Proliferation Assay, Promega) assay was performed as per manufacturer's instructions as a measure of osteoblast proliferation.

3.4 Osteoblast metabolic function

Human osteoblast cells (hFOB 1.19) were plated at 6×10^3 cells per well in an XF^e 24 Cell Culture Microplate (Seahorse Bioscience, USA). At confluency, the cells were stimulated with 0.35% PVI diluted in PBS or with PBS control for 3min, thoroughly washed as described previously and placed back into growth media. After 24 h at 33°C, XF Assay medium (XF base medium with 2mM GlutaMAX™) was then added to the plate and incubated at 33°C for 1 h.

Using a XF^e Extracellular Flux Analyzer (Seahorse Bioscience, USA) the oxygen consumption rate (OCR), extracellular acidification rate (ECAR) and proton production rate (PPR) of each well were measured simultaneously, in order to comprehensively profile the metabolic function of the osteoblasts.

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87 **3.5 Expression analysis of osteoblast mitochondrial oxidative phosphorylation** 88 **components**

89 Human osteoblast cells (hFOB 1.19) cells were seeded at 6×10^4 cells per well in a
90 24-well plate. Cells were stimulated with 0.35% dilute PVI or PBS control for 3min,
91 and then washed in PBS as previously described before being cultured in growth
92 media for 24 h (37°C, 5% CO₂). Following 24 h, cells were washed in PBS and lysed
93 in 1xLaemmli Sample Buffer (2%SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.002%
94 Bromophenol Blue, 0.0625M Tris HCL). Equal amounts of total protein lysates were
95 then applied to 12% SDS PAGE electrophoresis and blotted onto polyvinylidene
96 difluoride (PVDF) membrane at 150V for 1 h. Blots were immunoprobed with an
97 optimised antibody cocktail which contains antibodies to detect key components of
98 mitochondrial oxidative phosphorylation, namely CII-SDHB 30kDa (ab14714), CIII-
99 UQCRC2 (ab14745) and CV-ATP5A (ab14748). Blots were developed using ECL™
100 Prime Western Blotting Detection Reagent Kit (GE Healthcare, UK) on the
101 ChemiDoc™ Imaging MP System (BIO-RAD, USA).

103 **3.7 Osteoblast bone nodule formation and mineralization assay**

104 Human osteoblast cells (hFOB 1.19) were seeded at 6×10^4 cells per well in a 24 well
105 plate and treated with or without 0.35% PVI for 3 min as described previously, before
106 being cultured in differentiation media. After 14 days, cells were stained with alizarin
107 red solution in order to quantify the degree of mineralisation following the formation
108 of bone nodules. Briefly, cells were incubated in alizarin red staining solution (0.5%
109 Alizarin Red (Sigma-Aldrich, UK) in 1% ammonia solution at pH 4.5) for 10min at
110 room temperature and washed with PBS to remove excess stain. Cells were then
111 incubated in 10% cetyl pyridinium chloride (Sigma-Aldrich, UK) for 10 min at room

112 temperature. The supernatant was collected from each well and diluted 1:10 with the
113 10% cetyl pyridinium chloride and read at OD_{550nm} on a SpectraMAX Microplate
114 Reader (Molecular Devices, USA).

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116 **3.8 Statistical Analysis**

117 All statistical analyses were carried out using Prism5 (GraphPad Software Inc.
118 California, USA). Unless otherwise stated, all data within figures represents Mean
119 \pm SEM. MTS assay results were assessed by a one-way analysis of variance
120 (ANOVA) with a Tukey Post Hoc. OCR, ECAR and PPR, and alizarin red analysis
121 was compared using a Students un-paired t-test.

122 RESULTS

123 3.1 Acute exposure to PVI markedly reduces osteoblast proliferation.

124 Acute 3 min exposure of primary human osteoblasts to PVI at the clinical
125 concentration of 0.35% significantly decreased cellular proliferation at 48, 120 and
126 168 h post-exposure (Figure 1A). Expressed as a percentage of normal osteoblast
127 proliferation (PBS control), osteoblasts exposed to 0.35% PVI exhibited a $24 \pm 3.7\%$
128 decrease in proliferation after 24 h (Figure 1B). This was a sustained response,
129 which resulted in a $72\% \pm 2.3\%$ decline in cellular proliferation at 1 week. The
130 significant effect of PVI on cellular proliferation was also confirmed in the human
131 osteoblast cell line (hFOB 1.19) (Figure 1C & 1D), with a $26 \pm 3\%$ after 24 h, and a
132 $42\% \pm 7\%$ decline in proliferation relative to control after 6 days post-exposure.

133

134 3.2 Acute exposure of osteoblasts to dilute PVI ablates osteoblast basal 135 metabolic function.

136 Assessment of metabolic functional activity of osteoblasts (hFOB.1.19) 24 h post-
137 exposure to 0.35% PVI or PBS control showed a significant reduction in OCR
138 (0.32 ± 0.13 vs -0.05 ± 0.12 pmol/min/ μ g), ECAR (0.10 ± 0.07 vs 0.01 ± 0.02 mpH/min/ μ g)
139 and PPR (87.64 ± 18.78 vs 3.33 ± 7.24 pmol/min/ μ g) (Figure 2A-C) in osteoblasts that
140 had been exposed to 0.35% PVI for 3 min.

141

142 3.3 Acute exposure of human osteoblasts to PVI markedly reduces CIII-SDHB 143 protein expression, a key component of oxidative phosphorylation.

144 To identify if the effect of 0.35% PVI on osteoblast metabolic function was due to a
145 deleterious effect on mitochondrial function we then assessed the protein expression
146 of electron transport chain subunits (II, III and CV), involved in mitochondrial

147 oxidative phosphorylation. Human osteoblasts exposed to 0.35% PVI for 3 min
148 exhibited a marked reduction in protein expression of succinate dehydrogenase B
149 (CII-SDHB), and a moderate reduction in the protein expression of cytochrome b-c1
150 complex subunit 2 (CIII-UQCRC2), compared to PBS control exposed osteoblasts.
151 The protein expression of CV-ATP5A remained unchanged (Figure 2D).

153 **3.4 Acute stimulation with PVI reduces osteoblast mineralization**

154 We next determined whether the deleterious effects of 0.35% PVI on osteoblast
155 proliferation and metabolic functional activity impacted on the ability of osteoblasts to
156 form bone nodules and mineralise. At confluency, osteoblasts were stimulated with
157 0.35% PVI for 3 min, washed with PBS thoroughly and allowed to proliferate and
158 differentiate for a further 2 weeks in differentiation media. Alizarin red stained
159 mineralisation was quantified and showed that osteoblasts exposed to 0.35% PVI
160 exhibited significantly reduced bone nodule mineralisation (0.28 ± 0.05), compared to
161 control PBS exposed osteoblasts (0.37 ± 0.06) (Figure 3).

Discussion

PVI is advocated for wound irrigation following spinal surgery (8, 9), despite recent reports of its cytotoxicity in multiple cell types (10-13). Our *in vitro* study demonstrates that dilute PVI has fundamental deleterious effects on human osteoblast cells and their innate ability to mineralise.

Utilising primary human osteoblasts, we have demonstrated that acute exposure of osteoblasts to PVI for only 3 min, followed by thorough washing, is sufficient to profoundly inhibit cellular proliferation. Dilute PVI was found to significantly inhibit proliferation within the first 24 h following the 3 min exposure to PVI. However, perhaps of greater concern, our data also reveals that the negative effects on proliferation are fully sustained over a time-course of 7 days, resulting in a 72% inhibition of proliferation at this time point. This demonstrates that once exposed to dilute PVI the osteoblast cells do not recover their proliferative activity. Critically, we have also demonstrated that exposure to dilute PVI inhibits the innate functional ability of osteoblasts to form mineralised bone nodules.

Our data suggests that exposure to dilute PVI inhibits human osteoblast proliferation and mineralisation by preventing normal osteoblast metabolic function, since both basal oxygen consumption rate (a measure of aerobic mitochondrial respiration; (16)) and extracellular acidification rate and proton production rate (a measure of glycolytic metabolism; (16)) were ablated within 24 h post-exposure to 3 min of dilute PVI.

186 With regard to previous studies which have reported the effect of PVI on the
187 osteoblast, Kaysinger (1995) found that 5% Betadine (0.5% PVI) was toxic to
188 chicken osteoblasts (15). It is of note that the first non-toxic concentration reported
189 by Kaysinger was 5% Betadine (0.5% PVI). From their results it is therefore not
190 possible to ascertain where between 0.05% and 0.5% the toxic effects of PVI are
191 manifested (15). In the paper of Cheng et al. (8) the concentration of 0.5% PVI
192 appears to have been interpreted as the lowest toxic concentration and hence
193 guided their choice of 0.35% PVI for irrigation. We would argue that this was a
194 fundamental misinterpretation of the report of Kaysinger et al. We have now
195 confirmed that 0.35% PVI is indeed toxic to human osteoblasts.

196

197 Cabral et al (2007) assessed PVI exposure in human alveolar bone. They found 10%
198 and 5% PVI resulted in immediate cell death. 1% PVI resulted in rounding up and
199 cellular detachment (implied cell death), and that cellular proliferation was reduced at
200 concentrations of 0.5% PVI and higher. Cabral also reported a significant reduction
201 in Alkaline Phosphatase (AP) production at 0.05% and 0.2% PVI (14), indicative of
202 reduced mineralisation activity. Our findings are in agreement with this, since we
203 also found that PVI reduced osteoblast proliferation and have demonstrated a
204 significant reduction in the production of mineralised bone (as measured by Alizarin
205 red staining) with 0.35% PVI.

206

207 Infection rates in spinal surgery vary widely. For example, infection rate following
208 anterior cervical surgery is low, at around 0.1%, but infection rates can be up to 19%
209 in spinal deformity surgery (5, 17). Critically, the results of deep infection can be
210 devastating and occasionally fatal in patient groups with multiple co-morbidities.

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211 Clearly therefore, efforts must be taken to reduce spinal infection. A very informative
212 Best Practice Guideline in 2013 made a number of recommendations for the “high
213 risk” spinal surgical patients. These included pre-operative washing with
214 chlorhexidine, nutritional assessment, broad spectrum antibiotics and topical
215 vancomycin powder. It is noteworthy that PVI irrigation was not among the
216 recommendations (18).

217

218 In 2005 and 2006 two reports from the same centre described the utilization of
219 0.35% PVI lavage in spinal surgery (8, 9), and reported a clinically significant
220 reduction in infection rates from 2.9% without PVI lavage, to 0.5% with PVI lavage.
221 Clearly this is a commendable reduction in infection rates apparently attributable to
222 the PVI lavage. However, the goal of many spinal surgeries is the establishment of
223 solid bony fusion (union). Therefore, the potential negative effect of a given irrigate
224 on bone union must be considered alongside any positive benefit on spinal infection
225 rates.

226

227 Measurement of union (and non-union) are difficult to comprehensively determine
228 using Computed Tomography (CT) (Carreon et al. 2008). The only reliable method is
229 surgical exploration. In lumbar fusion surgery non-union rates are reported to be 10-
230 20%. In fusions of 4-levels or more, and in particular in adult degenerative scoliosis
231 the rate of non-union can be as high as 20- 60% (9, 19-21).

232

233 Our study identifies a potential pathway for impaired bony union following PVI lavage
234 in the clinical setting. The two reports from Cheng et al. (8) clearly state that on
235 radiographic analysis there was no adverse effect on fusion rates. However, it is

236 noteworthy that the patients in these studies were overwhelmingly 2-level or fewer
237 low-back fusions. These particular patients have a fairly predictable union rate of
238 around 80-90%. It is possible therefore that the deleterious effect of PVI on the
239 human osteoblast may have a greater clinical impact on longer fusions with higher
240 inherent risks of non-union. Additionally Chang et al. (9) used plain X-ray for
241 evaluation of bony fusion. This is not a reliable method for assessing bony fusion,
242 with only 68% accuracy in lumbar spine fusions (22).

243

244 Outside the scope of this report, but of importance to the surgeon considering using
245 PVI for irrigation of surgical wounds are descriptions of systemic complications
246 following iodine lavage. These include: significant systemic iodine absorption, acute
247 thyroid dysfunction, cardiovascular collapse, renal failure and death. Whilst these are
248 not immediately relevant to bone healing, it does suggest that iodine lavage is not a
249 universally benign phenomenon (23-27).

250

251 There are limitations to our study. It is not possible to replicate *in vitro* the entire
252 biological system involved in bone healing. We cannot replicate or ascertain what the
253 effect would be on the pluripotent stem cells that subsequently migrate to the site of
254 bony healing after irrigation. We have only assessed the effect on one cell type (the
255 osteoblast), we do not know what the effect of 0.35% PVI would be on other cell
256 types involved in bone healing such as fibroblasts, osteoclasts and platelets. We
257 also accept that although our primary osteoblasts were human, they were not
258 isolated from spinal bone. It is therefore conceivable that osteoblasts within spinal
259 bone could behave differently to osteoblasts from the appendicular skeleton. It is

260 reassuring however that our results are similar to those described by Kaysinger from
 261 chicken embryonic tibiae (15).

262
 263 In summary, we have demonstrated a clear deleterious effect of PVI to osteoblast
 264 proliferation and function and mineralisation. We suggest the advocated
 265 antimicrobial gains may be offset by the potential for impaired bone healing,
 266 particularly in long fusion procedures with higher non-union rates, where there is no
 267 clinical outcome data relating to PVI irrigation.

268

269 Abbreviations

270

271	PVI	Povidone-Iodine
272	PBS	Phosphate buffered saline
273	MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt
275	CII-SDHB	Succinate Dehydrogenase B
276	CIII-UQCRC2	ubiquinol-cytochrome c reductase core protein II
277	CV-ATP5A	ATP synthase

278

References

1. Stone PW. Economic burden of healthcare-associated infections: an American perspective. *Expert review of pharmacoeconomics & outcomes research*. 2009;9(5):417-22.
2. Dohmen PM. Economic Burden of Surgical Site Infections in Cardiac Surgery. *J Med Microb Diagn*. 2013;2(3).
3. Mackenzie WG, Matsumoto H, Williams BA, Corona J, Lee C, Cody SR, et al. Surgical site infection following spinal instrumentation for scoliosis: a multicenter analysis of rates, risk factors, and pathogens. *The Journal of bone and joint surgery American volume*. 2013;95(9):800-6, S1-2.
4. Reames DL, Smith JS, Fu KM, Polly DW, Jr., Ames CP, Berven SH, et al. Complications in the surgical treatment of 19,360 cases of pediatric scoliosis: a review of the Scoliosis Research Society Morbidity and Mortality database. *Spine (Phila Pa 1976)*. 2011;36(18):1484-91.
5. Duckworth AD, Mitchell MJ, Tsirikos AI. Incidence and risk factors for post-operative complications after scoliosis surgery in patients with Duchenne muscular dystrophy : a comparison with other neuromuscular conditions. *Bone Joint J*. 2014;96-B(7):943-9.
6. Smith JS, Shaffrey CI, Sansur CA, Berven SH, Fu KM, Broadstone PA, et al. Rates of infection after spine surgery based on 108,419 procedures: a report from the Scoliosis Research Society Morbidity and Mortality Committee. *Spine (Phila Pa 1976)*. 2011;36(7):556-63.
7. Glotzbecker BE, Yolin-Raley DS, DeAngelo DJ, Stone RM, Soiffer RJ, Alyea EP, 3rd. Impact of physician assistants on the outcomes of patients with acute myelogenous leukemia receiving chemotherapy in an academic medical center.

Journal of oncology practice / American Society of Clinical Oncology. 2013;9(5):e228-33.

8. Cheng MT, Chang MC, Wang ST, Yu WK, Liu CL, Chen TH. Efficacy of dilute betadine solution irrigation in the prevention of postoperative infection of spinal surgery. *Spine (Phila Pa 1976)*. 2005;30(15):1689-93.

9. Chang FY, Chang MC, Wang ST, Yu WK, Liu CL, Chen TH. Can povidone-iodine solution be used safely in a spinal surgery? *Eur Spine J*. 2006;15(6):1005-14.

10. Balin AK, Pratt L. Dilute povidone-iodine solutions inhibit human skin fibroblast growth. *Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]*. 2002;28(3):210-4.

11. Cooper ML, Laxer JA, Hansbrough JF. The cytotoxic effects of commonly used topical antimicrobial agents on human fibroblasts and keratinocytes. *The Journal of trauma*. 1991;31(6):775-82; discussion 82-4.

12. Van den Broek PJ, Buys LF, Van Furth R. Interaction of povidone-iodine compounds, phagocytic cells, and microorganisms. *Antimicrobial agents and chemotherapy*. 1982;22(4):593-7.

13. von Keudell A, Canseco JA, Gomoll AH. Deleterious Effects of Diluted Povidone-Iodine on Articular Cartilage. *J Arthroplasty*. 2013;28(6):918-21.

14. Cabral CT, Fernandes MH. In vitro comparison of chlorhexidine and povidone-iodine on the long-term proliferation and functional activity of human alveolar bone cells. *Clin Oral Investig*. 2007;11(2):155-64.

15. Kaysinger KK, Nicholson NC, Ramp WK, Kellam JF. Toxic effects of wound irrigation solutions on cultured tibiae and osteoblasts. *J Orthop Trauma*. 1995;9(4):303-11.

16. Pike Winer LS, Wu M. Rapid analysis of glycolytic and oxidative substrate flux of cancer cells in a microplate. *PLoS one*. 2014;9(10):e109916.
17. Bohl DD, Webb ML, Lukasiewicz AM, Samuel AM, Basques BA, Ahn J, et al. Timing of Complications Following Spinal Fusion Surgery. *Spine (Phila Pa 1976)*. 2015.
18. Vitale MG, Riedel MD, Glotzbecker MP, Matsumoto H, Roye DP, Akbarnia BA, et al. Building consensus: development of a Best Practice Guideline (BPG) for surgical site infection (SSI) prevention in high-risk pediatric spine surgery. *J Pediatr Orthop*. 2013;33(5):471-8.
19. Tsutsumimoto T, Shimogata M, Yoshimura Y, Misawa H. Union versus nonunion after posterolateral lumbar fusion: a comparison of long-term surgical outcomes in patients with degenerative lumbar spondylolisthesis. *Eur Spine J*. 2008;17(8):1107-12.
20. Koller H, Pfanz C, Meier O, Hitzl W, Mayer M, Bullmann V, et al. Factors influencing radiographic and clinical outcomes in adult scoliosis surgery: a study of 448 European patients. *Eur Spine J*. 2015.
21. Yamada K, Abe Y, Yanagibashi Y, Hyakumachi T, Satoh S. Mid- and long-term clinical outcomes of corrective fusion surgery which did not achieve sufficient pelvic incidence minus lumbar lordosis value for adult spinal deformity. *Scoliosis*. 2015;10(Suppl 2):S17.
22. Kant AP, Daum WJ, Dean SM, Uchida T. Evaluation of lumbar spine fusion. Plain radiographs versus direct surgical exploration and observation. *Spine (Phila Pa 1976)*. 1995;20(21):2313-7.

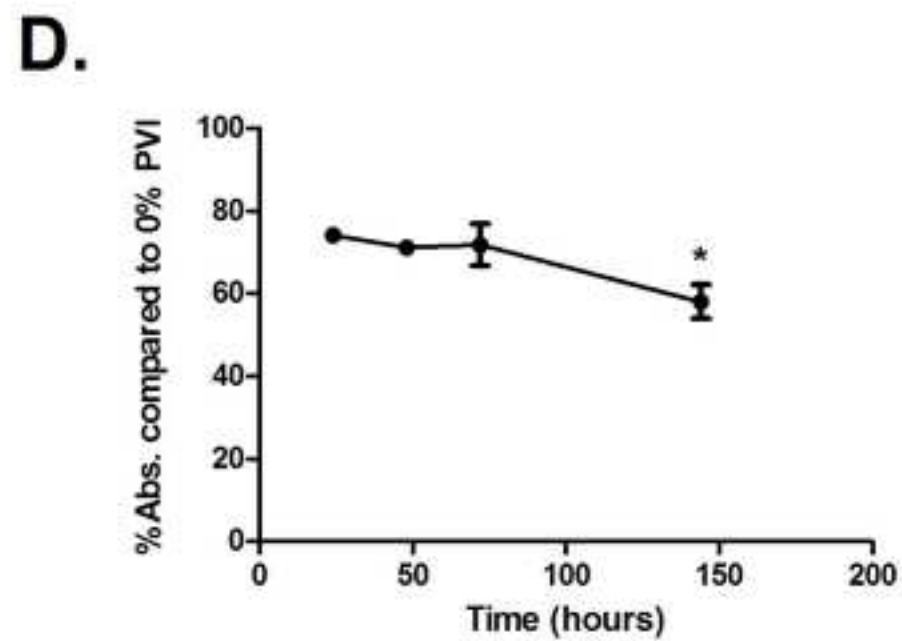
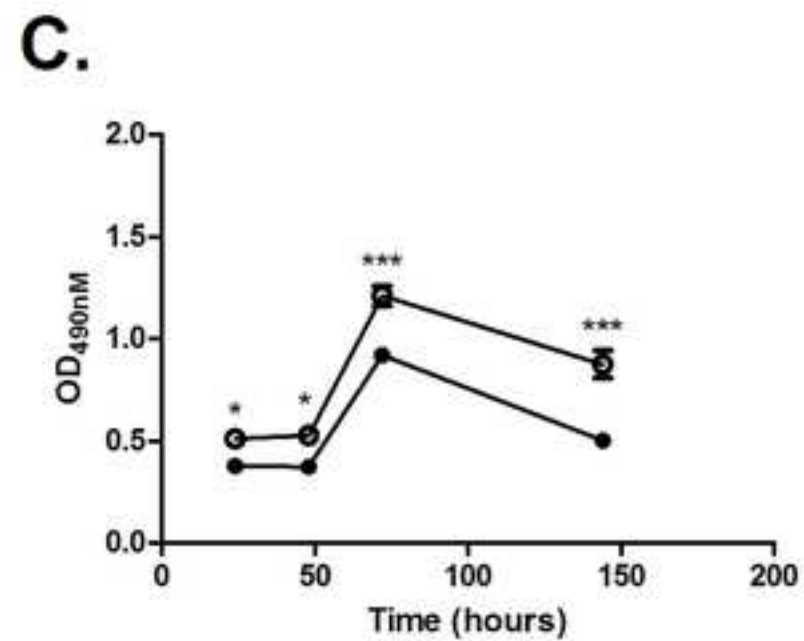
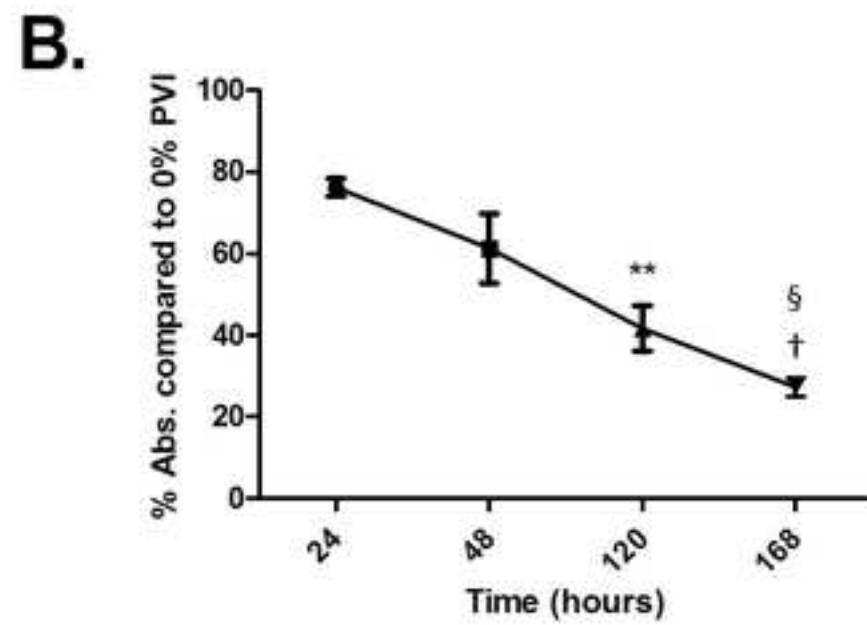
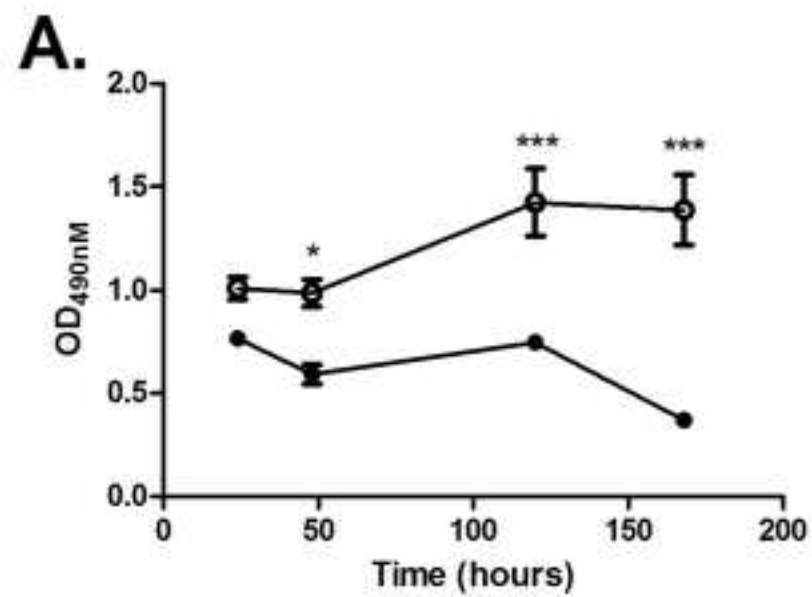
23. Campistol JM, Abad C, Nogue S, Bertran A. Acute Renal-Failure in a Patient Treated by Continuous Povidone-Iodine Mediastinal Irrigation. *J Cardiovasc Surg.* 1988;29(4):410-2.
24. D'Auria J, Lipson S, Garfield JM. Fatal iodine toxicity following surgical debridement of a hip wound: case report. *J Trauma.* 1990;30(3):353-5.
25. Aronoff GR, Friedman SJ, Doedens DJ, Lavelle KJ. Increased serum iodide concentration from iodine absorption through wounds treated topically with povidone-iodine. *Am J Med Sci.* 1980;279(3):173-6.
26. Rath T, Meissl G. Induction of hyperthyroidism in burn patients treated topically with povidone-iodine. *Burns Incl Therm Inj.* 1988;14(4):320-2.
27. Kurt TL, Morgan ML, Hnilica V, Bost R, Petty CS. Fatal iatrogenic iodine toxicity in a nine-week old infant. *J Toxicol Clin Toxicol.* 1996;34(2):231-4.

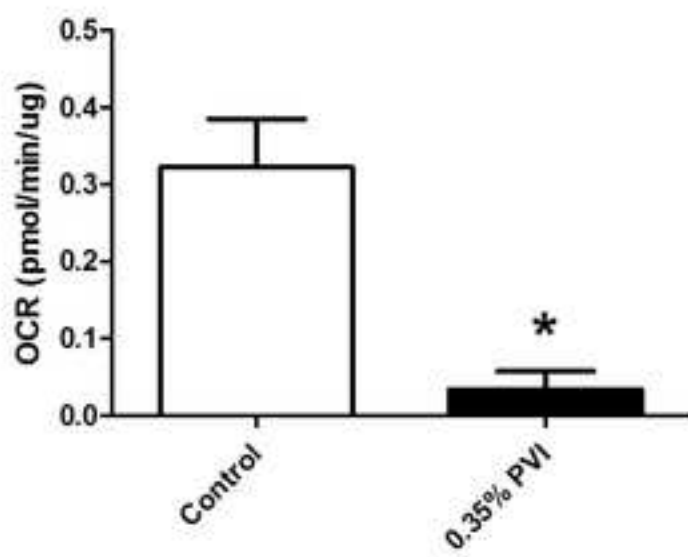
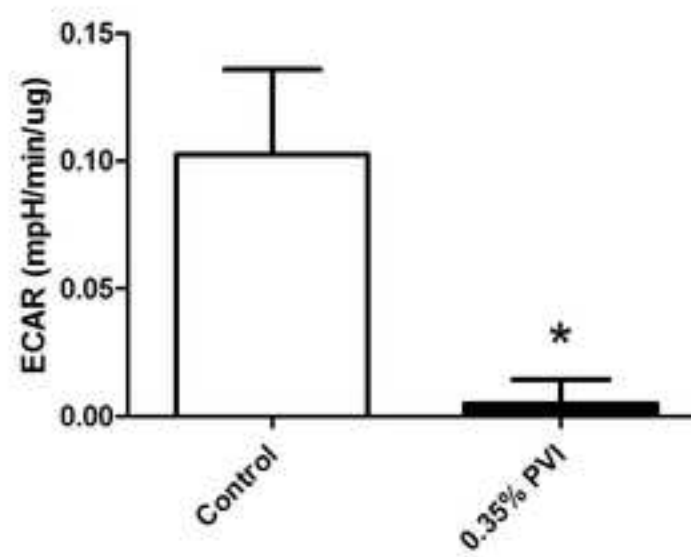
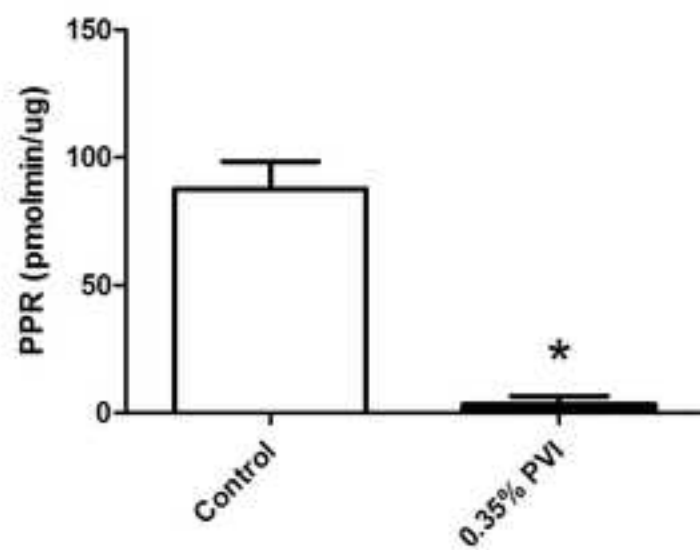
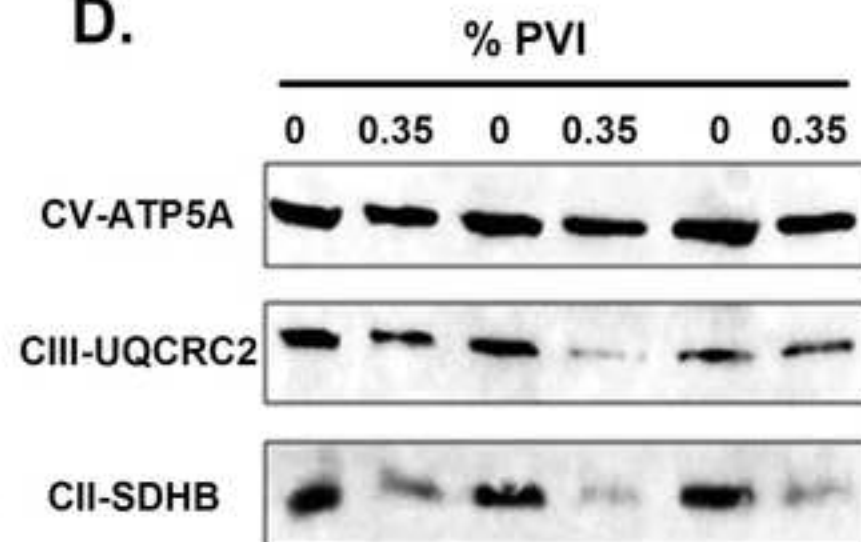
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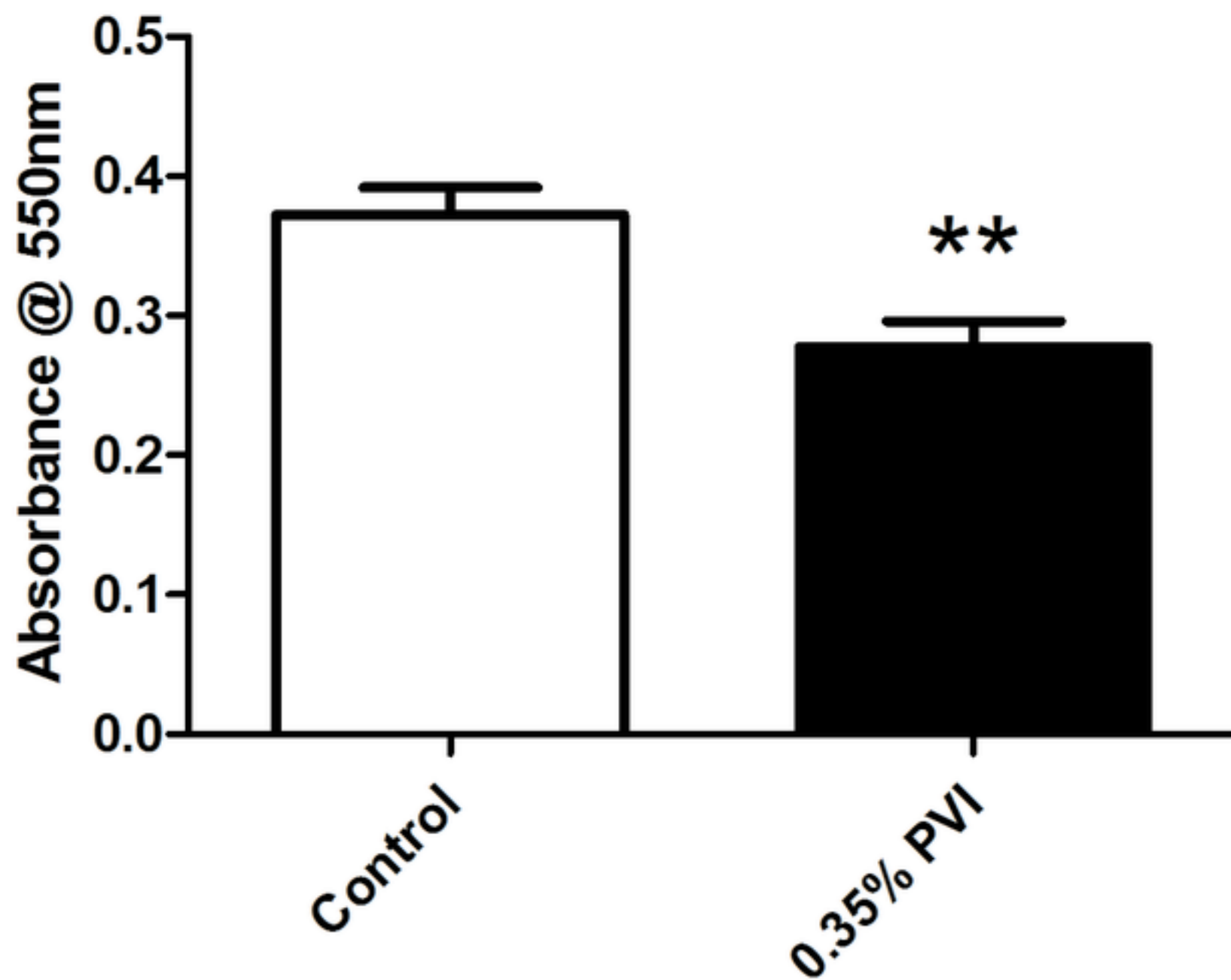
Figure 1. The effect of acute exposure to dilute PVI on the proliferation of primary human osteoblasts and a human osteoblast cell line. **A.** Primary human osteoblasts stimulated with PBS and 0.35% PVI for 3 minutes prior to time dependent MTS assays. ○ = PBS control; ● = 0.35% PVI stimulated (* $p < 0.05$, *** $p < 0.001$). **B.** Percentage proliferation of primary osteoblasts when compared to PBS control stimulated osteoblasts (** $p < 0.01$ between 24 and 120 h, § $p < 0.001$ between 24 and 168 h, † $p < 0.01$ between 48 and 168 h). **C.** hFOB 1.19 cell proliferation as measured by MTS assay (* $p < 0.05$, *** $p < 0.001$). **D.** Percentage proliferation of hFOB 1.19 osteoblasts when compared to PBS control stimulated osteoblasts (* $p < 0.05$; $n = 3$).

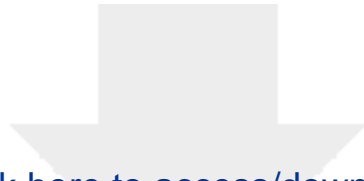
Figure 2. Metabolic activity of stable osteoblast culture following acute PVI stimulation. **A.** Oxygen consumption rate (OCR) of osteoblasts. **B.** Extracellular acidification rate of osteoblasts. **C.** Proton production rate of osteoblasts (Figure 2A-C $n = 4$, * $p < 0.05$). **D.** Western blot of cells stimulated with 0.35% PVI or PBS control 24 h prior to lysis and assayed for mitochondrial protein expression ($n = 3$).

Figure 3. Osteoblast mineralization following acute PVI stimulation. Alizarin red staining of osteoblasts treated with 0.35% PVI or PBS control was quantified at OD_{550nm} using 10% cetyl pyridinium chloride. ($n = 8$, ** $p < 0.01$).



A.**B.****C.****D.**

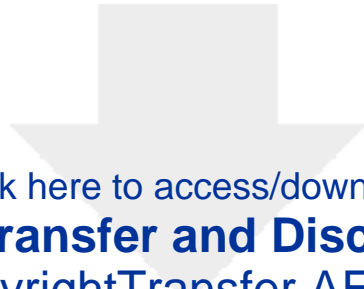




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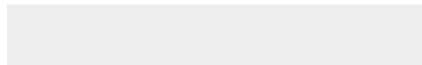
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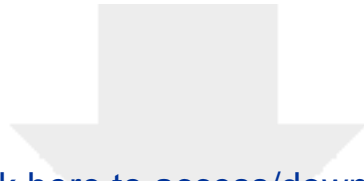




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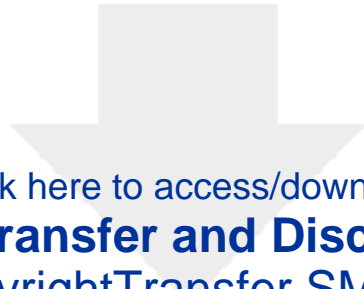




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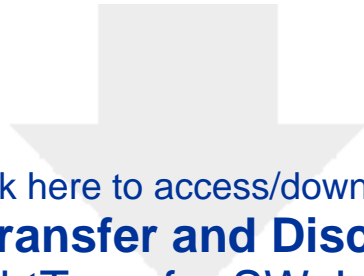




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